Center for Veterinary Biologics
and
National Veterinary Services Laboratories
Testing Protocol

Supplemental Assay Method for Scoring Feline Rhinotracheitis Virus in Cats Following Challenge

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Supplemental Assay Method for Scoring Feline Rhinotracheitis Virus in Cats Following Challenge

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1. Introduction

This Supplemental Assay Method (SAM) describes the method for determining the potency of feline rhinotracheitis virus (FRV), killed virus (KV) vaccines and the immunogenicity of FRV Master Seed Virus (MSV) by the vaccination and challenge of susceptible cats. Statistical weights are assigned to various clinical signs. A significant difference in scores between vaccinates and controls must be observed before the MSV or KV is considered to be satisfactory.

2. Materials

2.1 Equipment/instrumentation

2.1.1 Digital thermometer[1]

2.1.2 Centrifuge[2] with rotor[3]

2.1.3 Water bath[4] meeting the requirements of the current version of GDOCSOP0002

2.1.4 Atomizer[5] with compressed gas duster[6]

2.1.5 Self-refilling repetitive syringe, 2 ml[7]

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[1] Model M216, GLA Agricultural Electronics, 4120 Horizon Ln., San Luis Obispo, CA 93401 or equivalent
[2] Model J6B, Beckman Instruments, Inc., 2500 Harbor Blvd., Box 3100, Fullerton, CA 92834-3100 or equivalent
[3] Model JS-4.0 Beckman Instruments, Inc. or equivalent
[4] Cat. No. 15-461-10, Fisher Scientific Corp., 2000 Park Ln., Pittsburg, PA 15275 or equivalent
[5] Cat. No. 163, Sunrise Medical, 100 Devilbiss Dr., Somerset, PA 15501 or equivalent
[6] Cat. No. DPSR, Falcon Safety Products, Inc., 25 Chubb Way, Branchburg, NJ 08876 or equivalent
[7] Wheaton, Cat. No. 13-689-50C, Fisher Scientific Corp. or equivalent

2.2 Reagents/supplies

2.2.1 Use FRV-susceptible cats at the minimum age recommended for vaccination, shown to be negative for FRV serum neutralizing (SN) antibodies and negative for evidence of FRV. A cat shall be considered susceptible if a throat swab is negative by virus isolation and the serum is negative for SN antibody at a dilution of 1:2 in a 50% plaque reduction test or another SN test of equal sensitivity acceptable to the Animal and Plant Health Inspection Service (APHIS).

2.2.1.1 Numbers of animals required for testing:

1. Code of Federal Regulations, Title 9 (9 CFR) Part 113.211: 5 vaccinated cats (VC); 3 controls (CONT)

2. 9 CFR, Part 113.315(c)(1): 20 VC; 10 CONT

3. 9 CFR, Part 113.315(c)(4): 10 VC; 6 CONT or 5 VC; 3 CONT

2.2.2 FRV Challenge[8]

2.2.3 Minimum Essential Medium (MEM)

2.2.3.1 9.61 g MEM[9]

2.2.3.2 2.2 g sodium bicarbonate ($NaHCO_3$)[10]

2.2.3.3 Q.S. to 1000 ml with deionized water; adjust pH to 6.8-6.9 with 2N hydrochloric (HCL) acid.[11]

2.2.3.4 Sterilize through a 0.22-µm filter.[12]

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[8] Available upon request from the Center for Veterinary Biologics-Laboratory, P.O. Box 844, Ames, IA 50010 or another equivalent FRV challenge approved by APHIS
[9] MEM with Earle's salts without sodium bicarbonate, Cat. No. 410-1500EF, Life Technologies, Inc., 8400 Helgerman Ct., Gaithersburg, MD 20884 or equivalent
[10] Cat. No. S 5761, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178 or equivalent
[11] Cat. No. 9535-01, J.T. Baker, Inc., 222 Red School Ln., Phillipsburg, NJ 08865 or equivalent
[12] Cat. No. 12122, Gelman Sciences, 600 S. Wagner Rd., Ann Arbor, MI 48106 or equivalent

2.2.3.5 Store at 4° ± 2°C.

2.2.4 Transport Media

2.2.4.1 100 ml MEM

2.2.4.2 Aseptically add:

1. 300 units/ml penicillin[13]

2. 150 mg/ml gentamicin sulfate[14]

3. 300 mg/ml streptomycin[15]

4. 7.5 mg/ml amphotericin B[16]

2.2.5 Transport Media Tube

2.2.5.1 Pipette 2 ml of Transport Media into 12 x 75-mm polystyrene tubes[17] with the repetitive syringe.

2.2.5.2 Store at 4° ± 2°C.

2.2.6 Serum separation (Vacutainer®) tubes[18] and Vacutainer® needle, 20 ga x 1 in[19]

2.2.7 Syringe, 3 ml[20] and needle, 20 ga x 1 in[21]

2.2.8 Cotton-tipped swab[22]

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[13] Cat. No. 0049-0530-28, Schering Laboratories, 2000-T Galloping Hill Rd., Kenilworth, NJ 07033 or equivalent
[14] Gentocin® solution, Cat. No. 0061-0464-04, Schering Laboratories or equivalent
[15] Cat. No. S-9137, Sigma Chemical Co. or equivalent
[16] Cat. No. A 9528, Sigma Chemical Co. or equivalent
[17] Falcon 2058, Becton Dickinson Labware 1 Becton Dr., Franklin Lakes, NJ 07417-1885 or equivalent
[18] Vacutainer® 6514, Becton Dickinson Labware or equivalent
[19] Vacutainer® 5745, Becton Dickinson Labware or equivalent
[20] Luer-Lok®, Cat. No. 309585, Becton Dickinson Labware or equivalent
[21] Cat. No. 305175, Becton Dickinson Labware or equivalent
[22] Cat No. 14-960-3Q, Fisher Scientific Corp. or equivalent

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3. Preparation for the test

3.1 Personnel qualifications/training

Personnel must have training and experience in evaluating cats for clinical signs of disease due to FRV infection. **A current Animal Use Application (AUA) must be available prior to ordering the cats for the test.** All requirements in the AUA must be followed.

3.2 Preparation of equipment/instrumentation

On the day of challenge, set a water bath at 36° ± 2°C.

3.3 Preparation of reagents/control procedures

On the day of challenge, rapidly thaw the FRV Challenge in the water bath and dilute in MEM as recommended on the Center for Veterinary Biologics-Laboratory (CVB-L) Reference and Reagent Data Sheet. The FRV Challenge shall contain a minimum of 10^5 50% tissue culture infective dose ($TCID_{50}$) or plaque forming units (PFU).

3.4 Preparation of the sample

3.4.1 On the day(s) of vaccination, rehydrate a vial of the Test Serial, produced from the highest passage of MSV, according to the manufacturer's instructions with a 3-ml syringe and needle. Allow to incubate for 15 ± 5 min at room temperature (RT) (23° ± 2°C). No preparation of the KV is required.

3.4.2 Preparation of blood samples

3.4.2.1 Allow blood samples to clot in the Vacutainer® tubes at RT for 20 ± 5 min.

3.4.2.2 Separate serum from the clot by centrifuging the tubes at 1000 x *g* for 20 ± 5 min at 4° ± 2°C (2,200 rpm, Model J6B centrifuge with a JS-4.0 rotor).

3.4.2.3 Pour off the serum into labeled 12 x 75-mm polystyrene tubes.

3.4.2.4 Store serum samples at -20° ± 4°C until tested for SN antibodies.

3.4.3 Throat and nasal swab preparation

3.4.3.1 After collection of a throat and nasal swab, place in a labeled Transport Media Tube.

3.4.3.2 Store at -70° ± 5°C until tested by virus isolation.

4. Performance of the test

4.1 On the day of initial vaccination, cats are lightly sedated as per the AUA. Bleed, using the Vacutainer® system, from the jugular vein of both VC and CONT cats for SN antibody susceptibility determination. Swab the nasopharynx region and each nasal cavity of each cat using a cotton-tipped swab. Transfer the swab to a labeled Transport Media Tube, keeping each cat's swabs separate from the other cats.

4.2 Administer 1 dose of the Test Serial/KV as recommended on the label to all VC. Follow label recommendations for the interval between vaccinations if 2 doses are to be administered to the cats. **Note: CONT cats are not vaccinated.**

4.3 For -1 and 0 day postchallenge (DPC), determine and record rectal temperatures.

4.4 On the day of challenge, cats are lightly sedated as per the AUA. Using the atomizer, administer oral-nasally, 1.0 ml of the diluted FRV Challenge per cat during inhalation to both VC and CONT cats. Approximately 1/3 of the inoculum is administered in each nostril and the remaining 1/3 of the inoculum is administered directly into the posterior pharynx of the cat.

4.5 Observe cats daily from 1 to 14 DPC. Observe before and during feeding. Note and record all clinical observations. Scores are given for each day they occur as specified in **Appendix I**. In those instances where "each day" is not specified, scores are given only once, and the highest applicable score for each clinical sign is used.

4.6 After clinical observations are recorded, determine rectal temperatures and record from 1 to 14 DPC.

4.7 Data are analyzed so that a significant difference between VC (VC_i) and CONT (C_j) can be ascertained at the 0.05 level of significance (H_0: $VC_i=C_j$) using the Mann-Whitney modification of Wilcoxon's 2-sample test.

4.7.1 Total individual cat scores for the 14 DPC observation period.

4.7.2 Rank scores of all cats from the smallest to the largest. For identical ranks, assign the average to each cat, e.g. if the 3rd and 4th cat have an identical score, assign a rank of 3.5 to each cat. Retain identity of the rank as to whether it is a VC or CONT.

4.7.3 Total the ranking numbers of the CONT only; call this "T_1."

4.7.4 Compute $T_2 = C_N(C_N + VC_N + 1)-T_1$ where C_N = No. of CONT and VC_N = No. of VC.

5. Interpretation of the test results

5.1 The test is significant at the 0.05 level if:

T_2	**No. of cats in test**
$\geq$ 6	5 VC and 3 CONT
$\geq$ 32	10 VC and 6 CONT
$\geq$ 110	20 VC and 10 CONT

5.2 Validity requirements:

5.2.1 Cats are satisfactory for use in the test if the throat and nasal swabs are free of FRV and the serum samples are negative for antibody at a 1:2 dilution using a 50% plaque reduction test or SN test of equal sensitivity. Tests shall contain the minimum number of CONT cats, as stated in 9 CFR, Parts 113.211 or 113.315; otherwise the test is considered a no test and may be repeated.

5.2.2 9 CFR, Part 113.211(d)(2)(iv): At least 3 of 3 CONT cats shall show clinical sign of FRV, other than fever, otherwise the test is inconclusive and may be repeated.

5.2.3 9 CFR, Part 113.315(c)(3)(i): At least 8 of 10 CONT cats shall show clinical sign of FRV, other than fever, otherwise the test is inconclusive and may be repeated.

5.2.4 9 CFR, Part 113.315(c)(4)(i): At least 5 of 6 or 3 of 3 CONT cats shall show clinical sign of FRV, other than fever, otherwise the test is inconclusive and may be repeated.

5.3 If the cats meet the validity requirements in **Section 5.2** and T_2 is significantly different at the 0.05 level, the MSV/KV is satisfactory.

5.4 If the cats meet the validity requirements in **Section 5.2** and T_2 is not significantly different at the 0.05 level, the MSV/KV is unsatisfactory.

6. Report of test results

6.1 Record all test results on a test record.

7. References

7.1 Code of Federal Regulations, Title 9, Parts 113.211 and 113.315, U.S. Government Printing Office, Washington, DC, 1998.

7.2 Snedecor GW, Cochran WG. Statistical Methods, 6th ed. 1967;120.

8. Summary of revisions

8.1 This document was rewritten to meet the current NVSL/CVB QA requirements, to clarify practices currently in use in the CVB-L, and to provide additional detail. No significant changes were made from the previous protocol.

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9. Appendix I

CLINICAL SIGNS	DPC	SCORE
Fever		
103.0°-103.9°F (39.4°-39.9°C)		1 each day
104.0° to 104.9°F (40°-40.5°C)		2 each day
≥ 105.0°F (≥ 40.6°C)		3 each day
Conjunctivitis		
Serous discharge only	1-3	1
	≥ 4	2
Mucopurulent discharge	1-2	2
	3-5	4
	≥ 6	6
Rhinitis		
Serous discharge only	1-3	1
	≥ 4	2
Mucopurulent discharge	1-2	2
	3-5	4
	≥ 6	6
Sneezing		1 each day
Dyspnea		
Audible rales		2 each day
Coughing		2 each day
Open mouth breathing		3 each day
Depression		
Anorexia		1 each day
Dehydration	1-2	3
	≥ 3	4
Hypothermia [< 99°F (< 37.2°C)]		2 each day

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Oral ulcers (lingual or oral mucosa)		
1 ulcer (< 4 mm)	1-5	2
	6-9	3
	≥ 10	4
Multiple ulcers (< 4 mm)	1-4	3
	5-8	5
	≥ 9	7
Ulcers > 4 mm	1-4	5
	5-8	7
	≥ 9	9
Salivating		1 each day
External ulcers (lips or nares)		
Nonbleeding ulcer		4
Bleeding ulcer		6
Death		15